

Guidelines for Diet Control in Behavioral Studies

Behavioral research often requires that an animal perform a task for which it receives a food or fluid reward.^{1, 2, 5} This situation is not unlike conditions in the wild, in which animals must forage, travel extended distances, solve problems, or otherwise work to obtain their food and water. In the professional judgement of many investigators, veterinarians, and animal behaviorists, performing a task for rewards is behaviorally enriching for laboratory animals, especially nonhuman primates. The purpose of this document is to provide investigators with guidelines for the proper use of diet control in behavioral studies.

Food

Whenever an animal obtains any portion of its diet through food reward, the investigator must ensure that the sum of the nutritional value of the food earned through reward and of the food provided "free" (without the necessity of earning it) is sufficient to maintain the animal in a healthy state. Whenever possible, the food reward should be a "treat" (e.g., raisins, peanuts, flavored nutritionally balanced diets), which is sufficiently desirable and motivating for the animal that additional dietary restriction is minimized. However, dietary control is often required, depending on the species, the behavioral task, and the requirements of the research proposal.^{1, 3} In such cases, food should be provided every day, unless a specific exception to this policy has been obtained in an approved study proposal. Experience, however, has demonstrated that short periods (e.g. twenty-four hours) of markedly reduced or no food intake may be required during the initial phases of food control or after periods of increased food intake (e.g. ad libitum). To date, experience has demonstrated no adverse consequences to short periods (e.g. twenty-four hours) of no food intake in normal healthy animals. When caloric control is an experimental requirement, other aspects of the animals diet should remain balanced. Weight records must be kept on all animals on dietary control, a minimum of once each week, and should be available for examination by the veterinary staff and the ACUC. If an animal shows a loss in body weight of more than 15% of its weight at the start of the control period, the animal must be evaluated by a veterinarian and, if required its caloric intake increased appropriately. Young, developing animals have additional dietary requirements for maintaining their normal rate of growth, investigators working with young animals should specifically address this issue in their animal study proposal. Comparisons with litter mates or other similar control animals may prove useful when assessing young, developing animals. In some situations, when caloric restriction has been justified in developing animals, animals may never reach their projected adult size, but will in all other respects develop into normal adults. Exceptions to this policy will be allowed only if the attending or facility veterinarian determines that the weight loss does not endanger the health of the animal (e.g., in the case of an animal that was initially overweight, etc.). When transitioning an animal from a controlled food access paradigm to ad libitum access, careful monitoring of the animal's dietary intake of biscuits and fruits is recommended to aid in the prevention of deleterious gastrointestinal complications, e.g. bloat.

Fluid

As with food intake, whenever an animal obtains any portion of its fluid requirements through fluid rewards in behavioral testing, the investigator must ensure that the sum of the fluid earned through reward and the fluid provided "free" (without the necessity of earning it) is sufficient to maintain the animal in a healthy state. Experience has demonstrated that the transition of an animal to a controlled water access paradigm is best accomplished through a gradual, systematic limitation of fluid intake over a several day period.¹ Whenever possible, concurrent with the systematic limitation of available free choice water, animals should be provided with an opportunity to work for additional water until satiated.

In some institutions animals on controlled fluid paradigms are provided with "vacations". A "vacation" is a period of time, ranging from a day to a few weeks in duration, when the animal is provided a markedly increased fluid allocation, commonly ≥ 1.5 -3 times their routine daily consumption. When an animal is not required to perform their learned task for prolonged periods of time, several weeks duration or longer, gradually increasing the animal's consumption to ad libitum access is recommended. When transitioning an animal from a controlled water paradigm to ad libitum fluid access, careful monitoring of the animal's dietary intake of biscuits and fruits is recommended to aid in the prevention of deleterious gastrointestinal complications, e.g. bloat.

Experience has demonstrated that short periods (e.g. twenty-four hours) of markedly reduced or no fluid intake may be required during the initial phases of water control. Similarly, following a "vacation" period, an animal may require a period (\leq twenty-four hours) of no fluid intake, to again become motivated to perform their learned task. If periods of markedly reduced or no fluid intake are required, the principle investigator should provide a clear justification for the reduced fluid intake, as well as the extent and duration of fluid reduction in their animal study proposal. To date, experience has demonstrated no adverse consequences to short periods (e.g. twenty-four hours) with no fluid intake in normal healthy animals.⁴

Because the difficulty of the behavioral task at hand can, in part, determine the degree of water control required, whenever possible animals should be acclimated to easy task(s) prior to their systematic and gradual progression to more difficult paradigms. Once a baseline fluid intake has been established for a given animal on a given paradigm, the animal should be allowed to earn fluids to satiety or its fluid intake should be appropriately supplemented on a daily basis. In cases where supplements are required, the minimum amount of fluids to be provided each day should be equivalent to the amount typically consumed by the animal when it is permitted to earn fluids to satiety. It is recognized, however, that to ensure both the animal's welfare and experimental integrity, daily adjustments in fluid intake are often required during the course of the experimental paradigm. Once an animal has learned a behavior, the daily amount of fluid provided should be increased to the maximum level that will ensure

adequate performance of the task.

Assessment Of Adequacy Of Fluid Intake

Even though animals typically learn to work in a manner that earns their entire daily fluid requirement during the testing session, a number of precautions must be taken to avoid the detrimental effects of fluid control. The nature (e.g. water, fruit juice) and, if applicable, concentration of the fluid reward should be specified in the animal study proposal. Daily records of fluid intake must be maintained and be available for review by the veterinary staff and the ACUC. Each animal under fluid control must be observed daily for health status by the animal care staff. Normal physiological responses to fluid control routinely result in changes in the animal's clinical pathological status. For example, fluid control will often result in elevated blood parameters (e.g. Hct, TP, etc.), while physical and behavioral assessment of the animal indicates that the animal is healthy and adapting normally to the controlled access paradigm.^{1, 2} If at any time the attending veterinarian determines that an animal is not adapting sufficiently to the controlled fluid paradigm, the veterinarian will consult with the investigator to develop a plan to maintain the health of the animal.

Some animals on a controlled fluid access paradigm may decrease their total caloric intake in response to changes in their access to water. In most cases, the decreased caloric intake is minor and does not result in a body weight loss greater than fifteen percent (>15%). However, in the case of obese animals or those experiencing chronic fluid deficiency, loss of body weight in excess of 15% has been observed. This does not pose a problem in the case of the obese individual, but can lead to severe complications in the case of a chronic fluid deficiency. Therefore, as a precaution against chronic fluid deficiency, the animal's weight must be measured and recorded at no less than weekly intervals. If an animal shows a loss in body weight of more than 15% during the control period, the animal must be evaluated by a veterinarian and, if required its fluids increased appropriately. Exceptions to this policy will be allowed only if the attending or facility veterinarian determines that an animal is adequately hydrated and that the weight loss does not endanger the animal's health.

Summary: It is imperative that investigators, animal care staff and veterinarians working with animals on food or water controlled access paradigms know the species-specific stereotypic signs of distress for the animals they are working with. Animals routinely adapt well to the paradigm and display no signs of distress.¹ Animals must be carefully monitored on a daily basis to ensure that they are healthy, adapting normally and consume sufficient food/water to maintain their health status. Close monitoring is particularly important when an animal is initially acclimated to food or water control, during transition back to an ad lib state or when increasing the difficulty of the behavioral task. In all situations, the details of the training paradigm used and accountability of the individuals involved must be clearly outlined in the approved animal study proposal.

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